Application for Letters Patent

Multidimensional pump apparatus and Method for fully automated complex mixtures separation, identification, and quantification

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Background of the invention

Field:

The invention relates to high-pressure fluid pumping system that is capable of fluid delivery from nano- to analytical flow rates and more particularly to multidimensional HPLC instrumentation and method for the separation, identification, and quantification of complex mixtures such as cellular proteins and carbohydrates

State of the art:

Fluid pumping systems for high-pressure liquid chromatography (referred to hereinafter as HPLC) and the like are well know. In HPLC, complex sample with multiple components is transported through a column packed with particles of designed selectivity to cause separation of the components of the mixture when mobile solvent that is pumped through the column transports them. Typical prior art pumps employed in these applications were exemplified in U.S.patents Nos.4,045,343 to Achener et al, 4,260,342 to Leka et al, 3,599,045 to Gordon et al, and 5,253,981, 5,664,938, and 5,630706 to Yang et al. The micro-flow fluid pumping system invented by Yang et al is particularly important today for application in micro-LC-mass spectrometry (micro-LC-MS) in the separation and identification of biological samples. Today, the need for even lower flow rate fluid delivery at nano-liter/min range is emerging as the analysis of low abundance biological molecules such as cellular proteins and carbohydrates is growing in interest and important. Several LC instrument manufacturers have developed micro-HPLC pumping systems. The typical commercially available micro-HPLC systems are modified versions of conventional low pressure proportioning HPLC gradient pumps. See H. Bente, et al. U.S. Pat. No.4,714,545; G.Leka

al,U.S.Pat.No.4,260,342; P. Trafford et al, U.S. Pat. No. 4,728.434; P. Achener, et al, U.S. Pat. No.4,045,343; J. Rock , U.S. Pat. No. 4,128,476; H. Magnussen, Jr. U.S. Pat. No, 4,180,375; H. Magnussen, Jr, U.S. Pat. No. 4,131,393; and R. Allington, U.S. Patent No. 4,869.374. Such conventional systems use the split-flow technique (Sj. van der Wal et al., J. High Resolut Chromatogr. Comm. 6: 216, 1983), to obtain nl/min for nano-LC applications. Unfortunately, such split flow techniques are not reliable for routine applications at nano-flow rates as the flow restriction through the capillary column could change due to column inlet port blockage or flow restriction change inside the flow splitter. It is particularly difficult to maintain a constant split ratio when a high split ratio (for example 500µl/min to 0.1µl/min split i.e. 5000 to 1 split ratio) is required for nano-flow rate applications. An alternate approach for pumping in micro-HPLC is the single-stroke syringe-type piston pump (M. Munk, U.S.Pat. No.4.032,445; R. Brownlee, U.S. Pat.No,4,347,131; and R. Allington, U.S. Pat. No. 4,775,481. This type of syringe pump is capable of delivering solvent at a few µl/min. as limited by the number of steps per revolution of the stepping motor for driving the syringe piston. None of the existing HPLC apparatus can be used for flow rate ranges desirable in both splitless Nano/capillary column-HPLC at a flow rate range of 1 nl/min to 10ul/min and micro/analytical column-HPLC at a flow rate range of 10µl/min to 1,500µl/min. This invention addresses a pumping system that has dual flow rate delivery ranges without flow splitting and can perform both nano/capillary column-HPLC in a flow rate range between 1 nl/min to 10µl/min as well as micro/analytical-HPLC in a flow rate range between 1 µl/min to 1,500µl/min for separation, identification, and quantification of low abundance

biological samples such as cellular proteins and proteins reaction products. An example for the application of the nano-HPLC and Micro-HPLC dual flow rate ranges of this invention is shown for its usage in a fully automated on-line multi-dimensional HPLC-ICAT(Isotope-Coded Affinity Tag,(ICAT) Aebersold, R Gugi et al. Amercan Genomic Proteomic Technology, July/Aug 2001, 22-27) for cellular proteins separation, identification, and quantification.

As the inherent complexity of cellular proteins could be as much as several thousands and with a wide dynamic difference in abundance, it is necessary to develop the separation technique for rapid separation, identification, and quantification of those biological samples. The 2-Dimensional Gel Electrophoresis followed by direct analysis by tandem mass spectrometry of peptide mixtures generated by the digestion of complex protein mixtures has been proposed (Dongre A.R.et al., Trend Biotechnol.1977 15: 418-425). However, the 2-D gel electrophoresis method is difficult to automate and cannot detect low abundance proteins. Micro-LC-MS/MS has also been used successfully for separation and identification of proteins without gel electrophoresis separation (Opitek G.J. et al., Anal. Chem. 1997 69: 1518-1524). With the commercialization of capillary column HPLC and electrical spray/nano-spray interfaces for mass spectrometers, the interest for finding cancer cellular protein markers is growing significantly in the past four years. The invention of Goodlett et al (U.S. patent number 6,629,040) provides method and reagents for cellular proteins analysis. The method utilizes affinity-labeled protein reactive reagents that allow for selective isolation of peptide fragments or products of reaction with a given protein from complex biological mixtures. Isolated peptides or reaction products are identified by mass spectrometer (MS). In particular, the sequence of isolated

peptides can be determined using tandem MS techniques, and by applications of sequence database searching techniques, the protein from which the sequenced peptide originated can be identified. The reagents also provide for differential isotopic labeling of the isolated peptides or reaction products, which facilitates quantitative determination by mass spectrometry of the relative amounts of proteins in different samples. Also, the use of differentially isotope labeled reagents as internal standards facilitates quantitative determination of the absolute amounts of one or more proteins present in the sample.

The Isotope-Coded Affinity Tag (ICAT) chemistry as presented by Goodlett et al (U.S. patent number: 6,629,040) requires multiple off-line manual reaction processes. It cannot be practiced with small quantity of samples and is not automated. As a result, the method was not easily applicable to routine low abundance cellular proteins separation, identification, and quantification. This invention is to provide a fully automated multidimensional apparatus that allows on-line complex sample separation, reaction, cleavage, and nano-LC-MS-MS procedures. More specifically, the invention provides fully automated steps that utilizes multi-dimensional HPLC instrument and switching valves to fully automated ICAT™ (S.P. Gygi et al Nat. Biotechnol. 1999, 17, 994-999) , IMAC (Immobilized metal affinity chromatography (IMAC) (L. Andersson et al Anal biochem. 1986, 154(10), 250-254) multi-steps procedures and to allow reproducible multi-dimensional capillary/micro-bore column HPLC and mass spectrometry analysis of low abundance cellular proteins and protein reaction products.

Summary of the Invention:

The apparatus of the present invention comprises five elements.

The first element is the means for pumping liquid such as a HPLC that is designed to allow pumping capability from 1 nl/min to 1,500µl/min with or without flow splitting to ensure reliable and reproducible operations to meet the requirement of proteome analysis for clinical or diagnostic applications. The fluid pumping has high-speed two positions 6 port valve to allow reliable fluid channel switching to synchronize with the piston begin and end of a refill stroke. The second element is the combination of at least six or more of the first elements and reagents with valve switching capability for fully automated on-line ICAT™ or IMAC procedures. The third element is the utilization of multi-channel selection valves for parallel processing of multiple sample streams for sample trapping or collection. The forth element is the utilization of ion-exchange columns, reversed phase HPLC columns, affinity HPLC columns, size exclusion columns, metal chelating columns, Biotin cleavage columns, capillary reversed phase columns in series or in its combination to facilitated on-line ICAT, Immobilized metal affinity chromatography ((IMAC)Andersson, L; Anal. Biochem. 1986, 154 (1) 250-254), or other multiple complex sample analysis procedures that require more than three separation means or procedures. The fifth element is the use of tandem mass spectrometry and software for protein identification and quantification.

An example for the applications of the apparatus of the present invention is details in the following 12 steps procedures for ICAT™ or IMAC applications. The first step is to transport isotope labeled protein digest sample by pump 17 of the first element from an auto-sampler 25 or a HPLC injection valve 42 into an ion exchange 30 or size exclusion column. The second step is to stepwise or linearly increase the composition ratio of pump 18 (Salt) to pump 17 (H2O) of the first element for selectively eluting a portion of protein

digest sample from the SCX column 30 into the trap column 31. The step 3 is to pump reagent A (PH 7) by pump 19 of the first element to neutralize the trapping column 31. The step 4 is to switch valve 3 to allow the condition of the Avidin affinity column 3 to PH=7.2 with pump 19 of the first element. The step five is to elute Biotin labeled protein digest by pumping elution reagent using pump 20 of the first element into the Avidin affinity column 32. The step 6 is to elute non-cystein containing peptides to collection vial A from the avidin column 32 using pump 21 of the first element. The step 7 is to elute biotin labeled cystein-containing peptides into the cleavage column 33 by using pump 22 of the first element. The step 8 is to transport cleavage reagents A and B that is mixed in the auto-sampler 25 into the cleavage column 33 by using pump 17 of the first element. The step 9 is to cleave the biotin from cystein containing peptides in the cleavage column 33 at 37°C for 2 hours. The step 10 is the step while cleavage column 33 is in the cleavage step, the pump 17 of the first element is transporting the cleaved biotin to the waste 42 from cleavage column 33 that had completed steps 1 through 9 and ready for nano-LC-MS-MS. The step 11 is the step where the nano-LC-MS-MS at 50 to 2000nl/min solvent gradient flow rate with or without flow splitting was delivered by pump 23 and 24 of the first element to allow cystein containing isotope labeled peptides to be separated, identified and quantified by the nano-LC-MS-MS technique with the nano-trap column 35 and nano-LC column 36 in the preferred configuration as showed in the Figure 4. The step 12 is the step when the cleavage column 33 has completed 2 hours of cleavage step and the cleavage column 34 has completed nano-LC-MS-MS then the valves 28 and 29 switched to allow pumps 23 and 24 to elute biotin in the cleavage column 33 to waste 39 and to elute cystein-containing peptides for nano-LC-MS-MS. The valves 26 and 27 are also switched to allow peptides elution from the SCX column 30 to

the trap column 31 to repeat steps 1 through 12 again until the analysis of the whole protein digest is completed.

In the above step one; a high-pressure injection valve can be used to inject sample. The cleavage reagents A and B in the step 8 can also be pumped by two syringe pumps into a micro-mixer and then to the cleavage column(s). A multi-channel selection valve 8 (e.g. Valco 24 port multichannel selection valve) can be utilized to reduce the total analysis time of a complex sample. The apparatus includes the "multi-channel selection valve" as shown in Figure 12 to collect multiple fractions of eluted sample component from the Biotin affinity column into multiple arrays of cleavage columns or micro-plates.

Brief Description of Drawings:

Figure 1 is Block Diagram of a Close-Loop Digitally Controlled Syringe and Reciprocating Dual Modes Direct Drive Fluid Pump- Reciprocating Stroke Length Adjusted to 50% of Full Stroke for High Flow Rate Delivery---Reciprocating Fluid Pump

Figure 2 is a block diagram of a close-loop digitally Controlled syringe and reciprocating dual modes Direct drive fluid pump- reciprocating stroke end for high flow rate delivery-reciprocating fluid pump

Figure 3 is a block diagram of a close-loop digitally control syringe and reciprocating dual modes direct drive fluid Pump-----full stroke for single stroke syringe fluid pump

Figure 4 is a schematic of multi-dimensional system

Figure 5 is the flow diagram for step 1 process

Figure 6 is the flow diagram for step 2 process

Figure 7 is the flow diagram for step 3 process

Figure 8 is the flow diagram for step 4 process

Figure 9 is the flow diagram for step 5 process

Figure 10 is the flow diagram for step 6 process

Figure 11 is the flow diagram for step 7 process

Figure 12 is the flow diagram for multi-channel process

Figure 13 is the flow diagram for step 8 process
Figure 14 is the flow diagram for step 9 process
Figure 15 is the flow diagram for step 10 process
Figure 16 is the flow diagram for step 11 processes
Figure 17 is the flow diagram for step 12 processes

Detailed Description of the Invention:

Figure 1 is a simplified block diagram depicting key components of a preferred embodiment of a pumping system having a Servomotor 1 (or a step motor) with a gear box 2 of 50 to 1 ratio that has an optical encoder 3 mounted on the motor shaft for closed loop digital control for a motor 1. The motor 1 can be controlled to have one revolution in 40 to 80 minutes or longer. A total of 14,336,000 digital count is required to move the piston 7 linearly for 7cm. The piston 7 is a 0,159 cm diameter zirconium oxide that has piston volume of 150 µl. The motion of the piston 7 is controlled by proportion, integration and differentiation (PID) algorithm to ensure reproducible 0.5nl/digital count closed loop control. At 40 minutes per revolution or 1mm travel length, the minimum flow rate can be deliver with the servo motor 1 alone is 50 nl/min. The preferred embodiment of the invention having also a gear box 2 of 50 to 1 speed reduction factor to further increase the control accuracy to a flow rate delivery at 1 nl/min. The control accuracy allows nano-liter flow delivery without flow splitter. The invention further includes a preferred embodiment of a linear actuator 5 that is mounted to a bi-directional motor 1 and a piston holder 6, which is connected to a piston 7. An optical encoder 3 is mounted on the motor shaft for direct monitoring and controlling the gear motor. In a preferred embodiment, the optical encoder can be

mounted on a stepping motor or a servomotor with or without gear box 2. The optical encoder 3 is communicating to the controller interface module 15 which is in communication with a personal computer 16 to form closed loop digital control of the motor. The controller interface module 15 controls also the valve 10 on/off to synchronize with the beginning and the ending of the piston refill stroke. Figure 1 also showed a preferred embodiment where piston stroke length is set to 50% of the total stroke length to act as a reciprocating fluid pumping. In this example, the end of the fill stroke at 50% of the piston length 8 is detected by the optical encoder 3 when it reaches 50% of the digital counts, i.e. 7,168.000 counts for a 3.5cm stroke length. Valve 10 switched to close solvent entry from reservoir 9 and to allow solvent in piston chamber 11 to be delivered to column 14. In another preferred embodiment, a check valve 40 is installed at the outlet port of the piston chamber to ensure rapid pressurization of the piston chamber after refill stroke. The pressure transducer 12 indicates pump-head pressure. A micro-pulse dampener 13 is used to eliminate pump pulsation during refill operation. The end of column 14 is connected to a detector or mass spectrometer 15. In a preferred embodiment as shown in Figure 2, the optical encoder 3 provides closed loop digital counter to reach 14,336,000 counts for critical timing signal to switch valve 10 to open position at the end of piston stroke (i.e. 14,336,000 digital counts for a 7cm full stroke length in this example) to allow flow path opening between solvent reservoir 9 and the piston chamber 11. At the end of refill stroke as showed in Figure 3 where the piston 7 reaches its home port at location 8 in this example, the optical encoder 3 goes to zero count and again provides the critical timing signal to activate the valve 10 to close the flow path between the solvent reservoir 9 and piston chamber 11 and to open the flow path between the piston chamber 11 and the column 14 to

allow the solvent inside piston liquid chamber 11 to be pumped into the injector 42 and column 14. The invention further includes the software and/or firmware control of the pump head inlet switching valve 10 to open prior to the completion of the total digital counts for the forward motion (e.g. 14,336,000 digital counts for a 7cm full stroke length) to allow solvent refill into the piston chamber 8 and to switch pump head outlet valve 10 to open at the end of the refill stroke and after the closing of the pump head inlet valve. In the preferred embodiment, the pump head inlet valve and outlet valve is a 6-port Valco high speed-switching valve 10. The selection and manufacturers of the switching valve is not critical to the invention. A 4 port, 6 port, 10 port and others can also be selected for the switching purpose. For the purpose of switching speed of the valve, a switching time below 0.5 second is preferred. The mechanism of valve switching can be electrically actuated or by pneumatically activated.

Figure 3 shown also a preferred embodiment of this invention includes an optical encoder for closed loop digital counting for the beginning and ending of the piston stroke. In another preferred embodiment includes piston stroke length of 7cm or longer to facilitate single piston stroke fluid delivery without piston refill stroke interruption during an analysis. The stroke length of the piston 7 is configured with the pump control software to reset encoder digital counter to zero to cause the piston to refill upon the method download for nano-flow rate delivery. In another preferred embodiment, the software can also be programmed to allow rapid partial refill to a fraction of a total of 14,336,000 digital count measured by the optical encoder for reciprocating high flow rate delivery. The invention therefore produces a unique pumping system based on closed loop digital feed back control with

optical encoder to allow dual modes of solvent deliveries: the first mode is for nano-flow solvent delivery in syringe pump mode that can deliver from 1 nl/min to 10ul/min in single piston stroke upon method download. The second mode is to operate the pump as a partial stroke rapid refill reciprocating pump for continuous high flow rates delivery from 1 µl/min to 1,500µl/min for micro/analytical-HPLC

Referring to Figure 4 therein is shown a block diagram of a multi-dimensional apparatus embodiment of the invention including 8 HPLC pumps, 17,18,19,20,21,22,23,and 24 of the above described first element, four switching valves 26,27,28,29, one auto-sampler 25 or a time programmable injector 42, one strong cation exchange column 30, one reverse-phase trapping column 31, one affinity 32 (or metal chelating trap column), two cleavage columns 33 and 34, one capillary trap column 35 and one capillary analysis column 36, and one mass spectrometer 37, a controller interface module 15, and a computer 16 for method control and data handling. The system controller is a computer 16, which communicates with the system controller interface module 15 for editing and controlling every system components. The multiple pumping systems of 8 pumps in Figure 4 is a preferred means for multiple number of solvents delivery in the separation, trapping, cleavage, and LC-MS-MS based on the ICAT™ chemistry (S.P. Gygi et al Nat. Biotechnol. 1999, 17, 994-999). The specific number of pumps and means for the pump used for fluid deliver is not critical to the invention. The use of four or more switching valves in Figure 4 is a preferred means for column switching based on the ICAT™ chemistry (S.P. Gygi et al Nat. Biotechnol. 1999, 17, 994-999). The specific number of valves and the number of port on each valve is not critical to the invention and many commercially available switching valves can be used for the column switching means. The used of strong

cation exchange column 30, reversed phase trapping column 31, Avidin affinity column or metal affinity column 32, Cleavage columns 33 and 34, capillary reverse phase trapping column 35 and the nano-LC column 36 is the preferred means for complex sample separation based on ICAT™ chemistry (S.P. Gygi et al Nat. Biotechnol. 1999, 17, 994-999). The column 36 can be a capillary column with an integrated spray tip or a capillary column with a separated spray tip assembly. The 10-port valves 27 and 28 are preferred means for dual cleavage columns switching for double the speed of analysis. A four (4) or more than four (4) port switching valve can also be used for switching sample into multiple cleavage columns. The multi-channel switching valve 42 as showed in Figure 12 is also a preferred means for multiple sample fraction collection for sample eluting from the Avidin column 32. The specific number of the multichannel switching valve 42 in Figure 12 is not critical as long as it can be used to switch the flow path from the Avidin column 32 to allow multiple fraction collections. The specific column dimensions for columns 31,32,33,34,35,and 36 are not critical to this invention and can include capillary columns, packed capillary columns, micro-bore columns, packed columns, semi-pre columns, and pre-columns. The specific packing material and chemistry is not critical and can include reverse phases, normal phases, strong cation exchange particles, strong anion exchange particles, weak ion exchange particles, weak anion exchange particles, mix phases particles, affinity packing, metal affinity particles, size exclusion packing, chiral selectivity packing, and PH selectivity packing. Figure 4 shown also a preferred embodiment of an electro-spray or a nano-spray MS interface to introduce sample into ion source of a mass spectrometer. The specific brand name of manufacturers of the mass spectrometers is not critical and can include mass spectrometers manufactured by Thermo Electron Inc (Thermo-Finnigan), Applied

Biosystem Inc. (ABI), Brucker, Agilent, Perkin Elmer, Sciex, Leco, Varian, Beckmann, and others.

Figure 4 shown also a preferred embodiment including the fluid pumping system wherein a plurality of pumps each connected to separate solvent reservoirs containing different reagents in Applied Biosystem ICAT™ kit (Applied Biosystems ICAT Cleavable ICAT™ Reagent Methods Development Kit, Part number 4339035). In a preferred embodiment the system includes 8 independent capillary HPLC and nano-HPLC pumps that pairs up into 4 binary gradient HPLC for pumping 8 ICAT™ reagents for cellular proteins separation, identification, and quantification. Pump 17 is for cation exchange buffer-load (PH=3); pump 18 is for cation exchange buffer-elute (PH=3); pump 19 is for Affinity Buffer Wash 1 (PH=7.2); pump 20 is for Affinity Buffer-Load (PH=7.2); Pump 21 is for Affinity Buffer-wash 2 (PH=8.3); Pump 22 is for Affinity Buffer-Elute (PH=7.2); Pump 23 is for reverse phase LC-MS solvent 7(water with 0.3% to 0.5% formic or acetic acid); and Pump 24 is for reverse phase LC-MS solvent 8 (acetonitrile with 0.3% to 0.5% formic or acetic acid). The invention also includes four switching valves 26,27,28, and 29 for column selection for on-line performance of ICAT™ procedures. In a preferred embodiment a multi—channel selection valve 42 or a 10 port-switching valve 28 is used for multi-channel Biotin tag cleavage in steps 7 and 8.

Figure 5 is a preferred embodiment of flow diagram for the sample-loading step 1 with an autosampler 25. The sample can also be injected with a manual or electrical actuated injection valve 42. The sample is transported from the autosampler sample loop 43 of sample volume from 1 to 100ul into the ion exchange (SCX) column 30 (Micro-Tech Scientific, part # MC-10-SCX-300). The sample solvent and low retention components are then transported to the trapping column 31

where break through sample component is trapped. The sample solvent is then transported to waste 38. The ion exchange column 30 in this preferred embodiment has sufficient sample retention capacity to hold sample of interest within the column retention bed during sample injection process. The ion exchange column 30 can be a capillary column, a micro-bore column, an analytical column, a semi-preparative column, a preparative column, or a membrane trapping cartridge, a trapping disc, or a trapping pipette tip.

Figure 6 is a preferred embodiment of flow path of step 2 for eluting protein tryptic digest sample from the strong cation exchange column 30 to the trapping column 31 (Micro-Tech Scientific, Part # MC-5-C18-300) using ion exchange buffer-elute by pump 2. By controlling composition ratio of pump 18 to pump 17, the sample trapped in the ion exchange column 30 can be either partially in a controlled fractionation or completely eluted from the ion exchange column 30. The controlling gradient increment can be either stepwise or linearly time programmed. Pumps 17 and 18 in a preferred embodiment is a reciprocating pump at a flow rate from 1ul/min to 1,500ul/min.

Figure 7 is a preferred embodiment of flow path of the step 3 for removing salt and to condition the trapping column 31 to PH =7.2 using affinity buffer-wash 1 in pump 19. The switching valve 27 is at load position as shown in Figure 7.

Figure 8 is a preferred embodiment of flow path of step 4 for conditioning the Avidin column 32 (Applied Biosystems, part # 4326694) to PH =7.2. The valve 27 position is in injection position to allow affinity buffer= wash 1 solvent (PH=7.2) in pump 19 to remove salt from the Avidin column 32

Figure 9 is a preferred embodiment of flow path of the step 5 for pumping Affinity buffer-load (PH=7.2) to the avidin column 32 using affinity buffer-load in pump 20 to neutralize the Avidin columns.

Figure 10 is a preferred embodiment of flow path of the step 6 for removing non-labeled peptides using affinity buffer-wash 2 in pump 21. The eluent is then collected in vial A, 41

Figure 11 is a preferred embodiment of flow path of the step 7 for eluting labeled cystein containing peptides from the Avidin column 32 into the cleavage column 33 using affinity buffer-wash 2 in pump 22. A 10-port valve 28 can also be used for the collection of the cystein containing peptide in the cleavage column 33 (Micro-Tech Scientific, Part # MC-5-Zr2-300).

Figure 12 is also a preferred embodiment for using a multiple channel switching valve 42 as shown in Figure 12 for the collection of sample eluted from the Avidin column 32 in a array of collection vials, plates, or cleavage columns for further processing.

Figure 13 is the preferred embodiment for pumping the cleavage reagent A and B that is freshly prepared in the auto-sampler 25 into the cleavage column 33 using pump 17 as shown in step 8 where a column temperature is set to 37°C for Biotin cleavage from the cystein peptides.

Figure 14 is the preferred embodiment for depicting parallel operation capability of the system using a 10 port-switching valve to configure two cleavage columns 33 and 34. In the preferred embodiment as showed in the step 9, while the cleavage column 33 is in processing Biotin cleavage, the

system can also process nano-LC-MS-MS of the cystein containing peptides in the cleavage column 34 that had previously completed its biotin tag cleavage in the previous steps.

Figure 15 is the preferred embodiment the biotin tag to be removed to waste 44 from the cleavage column 33 by the reverse-phase HPLC solvent A in pump 23 when the valve 29 is in load position as shown in step 9. Figure 15 showed a preferred embodiment of connecting port 7 of the 10-port valve 28, an electrode, and a pre-column 35 (Micro-Tech Scientific, Part Number: MC-5-C18-300-CE) to the first Tee 45. The first Tee 45 can also be a cross. In this preferred embodiment the pre-column is then connected to a second Tee 46 where the analysis nano-LC column 36 and a waste solvent transfer line 39 are also connected. The outlet of the transfer line 39 is connected to a 6 port-switching valve 29 for flow stream diversion. In this particular arrangement the pump 23 transfers the biotin tag molecules from the cleavage column 34 into waste 39 when valve 29 is in load position. Cystein containing peptides however are trapped and concentrated at the pre-column 35.

Figure 16 is the preferred embodiment of nano-LC-MS-MS analysis of the cystein containing peptides trapped in the trap column 35 as shown in step 10. Wherein the sample is chromatographically separated in both the pre-column 35 and the nano-LC column 36 (Micro-Tech Scientific, Part Number: MC-15-C18W-75-CE) and then is sprayed into the mass spectrometer ionization source 37 for identification and quantification. In the preferred embodiment the valve 29 is in injection position to allow nano-LC elution of cystein peptides using reverse phase binary gradient generated by pump 23 and 24 in splitless mode at 0.1 to 1.0 ul/min flow rates.

Figure 17 is the preferred embodiment of the switching valves 26,27,28,29 as showed in step 11 to allow analysis of the cystein peptides in the cleavage column 33 at the end of the nano-LC-MS-MS analysis of sample in column 34. In this preferred embodiment, the sample in the ion exchange column 30 is again eluted into the trap column 31 to repeat steps 1 through 11 again until all peptides in the ion exchange column 30 have been completely eluted and analyzed.

Although the multi-dimensional pumping system and the closed loop digitally controlled gear motor driven pump are described primarily with reference to HPLC and ICAT™ analysis of cellular proteins, their uses are not limited to HPLC and ICAT™ applications (S.P. Gygi et al Nat. Biotechnol. 1999, 17, 994-999). The invention may include applications in proteome analysis, carbohydrates analysis, phosphoprotein isotope-coded affinity tag chemistry (M. B. Goshe et al, Anal Chem. 2001, 73, 2578-2586), Immobilized metal affinity chromatography (IMAC) (L. Andersson et al Anal biochem. 1986, 154(10), 250-254), and Multidimensional protein identification technology (MudPIT)(A.J.Link et al Nat. Biotechnol. 1999, 17, 676, W. H. McDonold et al Intern. J. Mass Spec 2002, 219, 245-251), capillary eletrophoresis, or other technology where multiple high pressure fluid delivery and valve switching and/or accurate, nano-flow rates, micro-flow rates, and analytical flow rates fluid delivery are necessary.

The Claims:

What is claimed is:

1. A fluid pump containing an optical encoder to facilitate digital closed loop control for synchronizing piston refill begin and end strokes with the opening and closing of electrically actuated or pneumatically actuated valve or